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AN   1998:77449  AGRICOLA
DN   IND21644440
TI   Purification of a novel type of SDS-dependent protease in maize using a
     monoclonal antibody.
AU   Yamada, T.; Ohta, H.; Masuda, T.; Ikeda, M.; Tomita, N.; Ozawa, A.; Shioi,
     Y.; Takamiya, K.
CS   Tokyo Institute of Technology, Yokohama, Japan.
AV   DNAL (450 P699)
SO   Plant and cell physiology, Jan 1998. Vol. 39, No. 1. p. 106-114
     Publisher: Kyoto, Japan : Japanese Society of Plant Physiologists.
     CODEN: PCPHA5; ISSN: 0032-0781
NTE  Includes references
CY   Japan
DT   Article
FS   Non-U.S. Imprint other than FAO
LA   English
AB   A protease which was activated by SDS was purified to homogeneity from
     maize leaves. On the basis of its proteolytic activity towards
     ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) or a synthesized
     peptide, the purification was carried out using immunoaffinity
     chromatography with a monoclonal antibody raised against a partially
     purified enzyme by native gradient PAGE. The purified
     protease showed three bands at 40, 15, and 13 kDa on SDS-PAGE, indicating
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that it was composed of heterogeneous subunits. The protease was specifically activated by SDS (optimum = 0.4% for Rubisco proteolysis), but not by poly-L-lysine, fatty acids, or ATP. The protease had a pH optimum around 4.9. beta-Mercaptoethanol stimulated the activity only in the presence of SDS. The proteolytic activity was sensitive to E-64 and leupeptin but was resistant to EDTA, suggesting that the enzyme was an SH-protease. Thus, this enzyme is a novel type of SDS-dependent protease which differs from proteasome, matrix metalloproteinase, and other proteases reported in many organisms.

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AN 1998:28102 AGRICOLA
DN IND20701807
TI Physical and kinetic evidence for an association between sucrose-phosphate synthase and sucrose-phosphate phosphatase.
AU Echeverria, E.; Salvucci, M.E.; Gonzalez, P.; Paris, G.; Salerno, G.
SO Plant physiology, Sept 1997. Vol. 115, No. 1. p. 223-227
Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-
CODEN: PLPHAY; ISSN: 0032-0889
NTE Includes references
CY Maryland; United States
DT Article; Conference
FS U.S. Imprints not USDA, Experiment or Extension
LA English
AB The possible formation of a multienzyme complex between sucrose (Suc)-phosphate synthase (SPS) and Suc-phosphate phosphatase (SPP) was examined by measuring the rates of Suc-6-phosphate (Suc-6-P) synthesis and hydrolysis in mixing experiments with partially **purified enzymes** from spinach (Spinacia oleracea) and rice (Oryza sativa) leaves. The addition of SPP to SPS stimulated the rate of Suc-6-P synthesis. SPS inhibited the hydrolysis of exogenous Suc-6-P by SPP when added in the absence of its substrate (i.e. UDP-glucose) but stimulated SPP activity when the SPS substrates were present and used to generate Suc-6-P directly in the reaction. Results from isotope-dilution experiments suggest that Suc-6-P was channeled between SPS and SPP. A portion of the SPS activity comigrated with SPP during native polyacrylamide gel electrophoresis, providing physical evidence for an enzyme-enzyme interaction. Taken together, these results strongly suggest that SPS and SPP associate to form a multienzyme complex.

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AN 1998:5046 AGRICOLA
DN IND20610087
TI Tonoplast ATPase from peanut seedlings.
AU Sen, S.; Sharma, V.
CS University of Roorkee, India.
SO Phytochemistry, June 1994. Vol. 36, No. 3. p. 569-572
Publisher: Oxford : Elsevier Science Ltd.
CODEN: PYTCAS; ISSN: 0031-9422
NTE Includes references
CY England; United Kingdom
DT Article
FS Non-U.S. Imprint other than FAO
LA English
AB Partially purified tonoplast ATPase from seven-day-old peanut seedlings shows a Km and Vmax value of 0.15 mM and 0.86 nkat Pi mg-1 protein, respectively. The enzyme is stimulated by Cl- and monovalent cations and inhibited by NO3(-). The enzyme is most effective with Mg2(+) ATP as the

substrate. The **purified enzyme** is highly unstable and requires phospholipids for activity. SDS-PAGE analysis of tonoplast ATPase shows it to be a multimeric structure of Mr 400000-600000 with Mr 69000, 55000 and 20000 major polypeptides. A polypeptide of 37000 is also present in minor amounts.

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AN 92:41715 AGRICOLA
DN IND92018783
TI A comparison of the allosteric properties of 6-phosophofructo-1-kinase from the flight muscle, leg muscle, mid-gut and hind-gut of the grasshopper, Poekilocerus bufonius.
AU Khoja, S.M.
AV DNAL (QP501.C6)
SO Comparative biochemistry and physiology : B : Comparative biochemistry, 1991. Vol. 99, No. 4. p. 833-837
Publisher: Oxford : Pergamon Press.
CODEN: CBPBB5; ISSN: 0305-0491
NTE Includes references.
DT Article
FS Non-U.S. Imprint other than FAO
LA English
AB Allosteric properties of 6-phosphofructo-1-kinase (PFK) from the grasshopper, P. bufonius, were compared among the night muscle, leg muscle, mid-gut and hind-gut after being partially purified with Sephadex G-100. The partially **purified enzyme** from the four tissues displayed cooperativity with respect to fructose 6-phosphate at pH 7.0, and was markedly inhibited by high concentrations of ATP. The activities of PFK were readily inhibited by phosphoarginine, glucose 1,6-bisphosphate and phosphoenol pyruvate, but were only slightly inhibited by citrate. The enzyme activities were stimulated by fructose 2,6-bisphosphate (Fru-2,6-P2), AMP, ADP and Pi with Fru-2,6-P2 being the most potent stimulator. The present results suggest that PFK prepared from the different tissues of the grasshopper, P. bufonius, is highly regulated with positive and negative effectors which allow the glycolytic flux to meet the energy demands either for jumping or flying.

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AN 2004:1059 BIOSIS
DN PREV200400003130
TI Purification and characterization of a fibrinolytic enzyme produced from Bacillus amyloliquefaciens K42 isolated from Korean soy sauce.
AU Yun, Gyung-Hyun; Lee, Eun-Tag; Kim, Sang-Dal [Reprint Author]
CS Department of Applied Microbiology, Yeungnam University, Kyongsan, 712-749, South Korea
sdkim@yumail.ac.kr
SO Korean Journal of Microbiology and Biotechnology, (September 2003) Vol. 31, No. 3, pp. 284-291. print.
ISSN: 1598-642X (ISSN print).
DT Article
LA Korean
ED Entered STN: 17 Dec 2003
Last Updated on STN: 17 Dec 2003
AB Bacillus amyloliquefaciens K-42, which produces strongly a fibrinolytic enzyme, was isolated from Ganjang, a traditional Korean soy sauce. The fibrinolytic enzyme was purified to homogeneity by ammonium sulfate fractionation, ion-exchange chromatography on DEAE-Sephadex A-50, gel chromatography on Sephadex G-100, and gel chromatography on Sephadex G-75 of the culture filtrate of Bacillus amyloliquefaciens K42. The **purified enzyme** showed the specific activity of 59.4 units per milligram, which was increased by 17.1 fold over the culture

broth. And the molecular weight of purified fibrinolytic enzyme was confirmed to be about 45,000 Dalton by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The enzyme activity was relatively stable at pH 4.0-10.0 and the optimum pH was 8.0. The activity of the **purified enzyme** was increased by Mg2+, Cu2+ but the enzyme was totally inhibited by Ba2+, Hg2+. In addition, the enzyme activity was potently inhibited by EDTA, EGTA and CDTA. It was concluded that the **purified enzyme** was a metalloprotease. And Km value was 2.03 mg/ml to fibrin.

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AN 2002:340858 BIOSIS
DN PREV200200340858
TI Purification and characterisation of a glutaminase from Debaryomyces spp.
AU Dura, M. A.; Flores, M.; Toldra, F. [Reprint author]
CS Instituto de Agroquimica y Tecnologia de Alimentos, CSIC, Burjassot, 46100, Valencia, Spain
ftoldra@iata.csic.es
SO International Journal of Food Microbiology, (5 June, 2002) Vol. 76, No. 1-2, pp. 117-126. print.
CODEN: IJFMDD. ISSN: 0168-1605.
DT Article
LA English
ED Entered STN: 12 Jun 2002
Last Updated on STN: 12 Jun 2002
AB A glutaminase was purified from the cell-free extract of Debaryomyces spp. CECT 11815 by protamine sulphate treatment and several chromatographic procedures including anion exchange chromatography and gel filtration. The **purified enzyme** consisted of two subunits, with molecular masses of 65 and 50 kDa, respectively. Activity was optimal at 40 degreeC and pH 8.5, and the Km value for L-glutamine was 4.5 mM. The glutaminase exhibited activity against L-gamma-Glu-methyl ester, L-gamma-Glu-hydrazide, and L-albiziin, while L-asparagine, CBZ-L-Gln, CBZ-L-Gln-Gly, glutathione, L-gamma-Glu-pNA and L-gamma-Glu-AMC were not hydrolysed. The enzyme was not affected by PMSF, DTT and EDTA. However, the enzyme was inhibited by sulfhydryl group reagents, DON, L-albizziin, L-asparagine and high concentrations of L-glutamine and ammonium, while L-aspartate did not affect the activity. Phosphate and acetate did not produce any significant effect on the glutaminase activity, but it was slightly stimulated by lactate and borate.

L4 ANSWER 7 OF 30 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:10023 BIOSIS
DN PREV200200010023
TI Purification and characterization of a fibrinolytic enzyme from Bacillus sp. KDO-13 isolated from soybean paste.
AU Lee, Si-Kyung; Bae, Dong-Ho [Reprint author]; Kwon, Tae-Jong; Lee, Soo-Bok; Lee, Hyung-Hoan; Park, Jong-Hyun; Heo, Seok; Johnson, Michael G.
CS Department of Applied Biology and Chemistry, Konkuk University, Seoul, 143-701, South Korea
donghoya@kkucc.konkuk.ac.kr
SO Journal of Microbiology and Biotechnology, (October, 2001) Vol. 11, No. 5, pp. 845-852. print.
ISSN: 1017-7825.
DT Article
LA English
ED Entered STN: 28 Dec 2001
Last Updated on STN: 25 Feb 2002
AB A microorganism producing fibrinolytic enzyme was isolated from Korean traditional soybean paste and identified as Bacillus sp. KDO-13. The fibrinolytic enzyme was purified to homogeneity by ammonium sulfate fractionation, ion-exchange chromatography on DEAE-celluose, and gel chromatography on Sephadex G-100 of the culture supernatant of Bacillus sp. KDO-13. The molecular weight of the **purified**

enzyme was estimated to be 44,000 by SDS-PAGE. The optimum pH and temperature for the enzyme activity were pH 8.0 and 50degreeC, respectively. The enzyme activity was relatively stable at pH 7.0-9.0 and temperature below 50degreeC. The activity of the enzyme was inhibited by Al3+ and Hg2+, but activated by Co2+ and Ni2+. In addition, the enzyme activity was potently inhibited by EDTA and o-phenanthroline. The **purified enzyme** could completely hydrolyze a fibrin substrate within 6 h in vitro, and had a low Km value for fibrin hydrolysis. It was concluded that the **purified enzyme** was a metalloprotease with relatively high specificity for fibrinolysis, and thus, could be applied as an effective thrombolytic agent.

L4 ANSWER 8 OF 30 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1979:208750 BIOSIS
DN PREV197968011254; BA68:11254
TI BLOCKADE BY N METHYL HYDROXYLAMINE OF ACTIVATION OF GUANYLATE CYCLASE EC-4.6.1.2 AND ELEVATIONS OF CYCLIC GMP LEVELS IN NERVOUS TISSUES.
AU DEGUCHI T [Reprint author]; SAITO M; KONO M
CS DEP MED CHEM TOKYO METROP INST NEUROSCI, 2-6 MUSASHIDAI, FUCHU, TOKYO 183, JPN
SO Biochimica et Biophysica Acta, (1978) Vol. 544, No. 1, pp. 8-19.
CODEN: BBACAQ. ISSN: 0006-3002.
DT Article
FS BA
LA ENGLISH
AB Hydroxylamine and N-methylhydroxylamine prevented the activation of soluble guanylate cyclase (EC 4.6.12.) by the endogenous activator as well as by nitroso compounds such as N-methyl-N'-nitro-N-nitrosoguanidine or nitroprusside, while other derivatives of hydroxylamine were ineffective. Hydroxylamine and N-methylhydroxylamine did not alter the basal guanylate cyclase activity of **purified enzyme** preparations.
Kinetics analysis indicated that N-methylhydroxylamine competed with N-methyl-N'-nitro-N-nitrosoguanidine for guanylate cyclase. The activation of guanylate cyclase by N-methyl-N'-nitro-N-nitrosoguanidine and its inhibition by N-methylhydroxylamine were reversible reactions. These effects of N-methyl-N'-nitro-N-nitrosoguanidine and N-methylhydroxylamine were observed with guanylate cyclase from rat brain, liver and lung. N-Methylhydroxylamine prevented the increase of cyclic[c]GMP levels in cerebellar slices of guinea pig by N-methyl-N'-nitro-N-nitrosoguanidine, veratridine and adenosine, while the elevations of AMP by these agents were not affected. N-methyl-hydroxylamine also blocked the increases of cGMP levels by carbachol, prostaglandin E1 and N-methyl-N'-nitro-N-nitrosoguanidine in mouse neuroblastoma N1E 115 cells. N-methylhydroxylamine prevented the activation of guanylate cyclase and the increased synthesis of cGMP in response to transmitters without blocking the synthesis of cGMP via basal enzyme activity.

L4 ANSWER 9 OF 30 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
AN 2000:30674640 BIOTECHNO
TI Overexpression in Escherichia coli and characterization of the chloroplast fructose-1,6-bisphosphatase from wheat
AU Tang G.-L.; Wang Y.-F.; Bao J.-S.; Chen H.-B.
CS H.-B. Chen, State Key Lab. of Bio-organ., Shanghai Inst. of Organ. Chem., Chinese Academy of Sciences, 354 Feng Lin Lu, Shanghai 200032, China.
E-mail: hbchen@pub.sioc.ac.cn
SO Protein Expression and Purification, (2000), 19/3 (411-418), 35 reference(s)
CODEN: PEXPEJ ISSN: 1046-5928
DT Journal; Article
CY United States
LA English
SL English
AB An important Calvin cycle enzyme, chloroplast fructose-1,6-bisphosphatase

(FBPase) from wheat, has been cloned and expressed up to 15% of the total cell protein using a pPLc expression vector in Escherichia coli by replacing the codons in the 5'-terminal encoding sequence with optimal and A/T-rich ones. The overexpressed wheat FBPase is soluble, fully active, and heat stable. It can be purified by chromatography in turn on DEAE-Sepharose and Sephacryl S-200, and around 15 mg of **purified enzymes** (>95%) is obtained from 1 liter of cultured bacteria. Its special activity is8.8 u/mg, K(cat) is 22.9/S, K(m) is 121 .mu.M, and V(max) is 128 .mu.mol/min .midldot. mg. The recombinant FBPase can be activated by DTT, Na.sup.+, or low concentrations of Li.sup.+, Ca.sup.2.sup.+, Zn.sup.2.sup.+, GuHCl, and urea, while it can be inhibited by K.sup.+ or NH.sub.4/.sup.+. (C) 2000 Academic Press.

L4 ANSWER 10 OF 30 CABA COPYRIGHT 2004 CABI on STN
AN 2003:96820 CABA
DN 20033072987
TI Purification and some properties of exo-1,4-[beta]-glucanase from Chaetomium olivaceum
AU El-Gindy, A. A.; Saad, R. R.; Fawzi, E.
CS Dept. of Biological Sciences, Fac. of Education, Cairo, Egypt.
SO Acta Microbiologica Polonica, (2003) Vol. 52, No. 1, pp. 35-44. 19 ref.
Publisher: Polskie Towarzystwo Mikrobiologow. Warsaw
ISSN: 0137-1320
CY Poland
DT Journal
LA English
ED Entered STN: 20030606
Last Updated on STN: 20030606
AB Exo-1,4-[beta]-glucanase (E.C. 3.2.1.91 [cellulose 1,4-[beta]-cellobiosidase]) was successively purified by precipitation with acetone, followed by gel filtration on Sephadex G-100 and chromatographed onto DEAE-cellulose. A typical procedure provided 47.14 fold purification with 72.8% yield. The molecular mass of the **purified enzyme** was found to be 88 kDa by SDS-PAGE. The pH optimum of the enzyme was 5.2 and maximum activity was obtained at 45[deg]C. Km value against [alpha]-cellulose was 0.65 mg ml-1. [alpha]-cellulose and filter paper were the best substrates for enzyme activity. Enzyme was activated by Mn2+ and Fe3+, inactivated by Cu2+ and completely inhibited by Hg2+ and Ag+.

L4 ANSWER 11 OF 30 CABA COPYRIGHT 2004 CABI on STN
AN 2003:93621 CABA
DN 20033066116
TI Plant C-N hydrolases and the identification of a plant N-carbamoylputrescine amidohydrolase involved in polyamine biosynthesis
AU Piotrowski, M.; Janowitz, T.; Kneifel, H.
CS Department of Plant Physiology, Ruhr-Universitat, Universitatsstrasse 150, D-44801 Bochum, Germany. Markus.Piotrowski@ruhr-uni-bochum.de
SO Journal of Biological Chemistry, (2003) Vol. 278, No. 3, pp. 1708-1712. 20 ref.
Publisher: American Society for Biochemistry and Molecular Biology Inc. Bethesda
ISSN: 0021-9258
CY United States
DT Journal
LA English
ED Entered STN: 20030606
Last Updated on STN: 20030606
AB A nitrilase-like protein from Arabidopsis thaliana (NLP1) was expressed in Escherichia coli as a His6-tagged protein and purified to apparent homogeneity by Ni2+-chelate affinity chromatography. The **purified enzyme** showed N-carbamoylputrescine amidohydrolase activity, an enzyme involved in the biosynthesis of polyamines in plants and bacteria. N-carbamoylputrescine amidohydrolase activity was confirmed by the identification of two of the three occurring products, namely putrescine

and ammonia. In contrast, no enzyme activity could be detected when applying various compounds, including nitriles, amines, and amides as well as other N-carbamoyl compounds, indicating the specificity of the enzyme for N-carbamoylputrescine. Like the homologous [beta]-alanine synthases, NLP1 showed positive cooperativity toward its substrate. The native enzyme had a molecular mass of 279 kDa, as shown by blue-native polyacrylamide gel electrophoresis, indicating a complex of eight monomers. The expression of the NLP1 gene was found in all organs investigated, but it was not induced upon osmotic stress, which is known to induce biosynthesis of putrescine. This is the first report of cloning and expression of a plant N-carbamoylputrescine amidohydrolase and the first time that N-carbamoylputrescine amidohydrolase activity of a recombinant protein could be shown in vitro. NLP1 is one of the two missing links in the arginine decarboxylase pathway of putrescine biosynthesis in higher plants.

L4 ANSWER 12 OF 30 CABA COPYRIGHT 2004 CABI on STN
AN 2002:67369 CABA
DN 20023024479
TI Purification and characterization of urease isolated from the pathogenic fungus Coccidioides immitis
AU Mirbod, F.; Schaller, R. A.; Cole, G. T.
CS Department of Microbiology and Immunology, Medical College of Ohio, 3055 Arlington Avenue, Toledo, OH 43614-5806, USA. gtcole@mco.edu
SO Medical Mycology, (2002) Vol. 40, No. 1, pp. 35-44. 32 ref.
Publisher: BIOS Scientific Publishers Ltd. Oxford
ISSN: 1369-3786
CY United Kingdom
DT Journal
LA English
ED Entered STN: 20020405
Last Updated on STN: 20020405
AB Coccidioides immitis, the causative agent of San Joaquin Valley fever (coccidioidomycosis), produces a urease which has been suggested to contribute to the virulence of this fungal pathogen. Urease catalyses the hydrolysis of urea and has been proposed to at least partly account for alkalinity of the microenvironment in which C. immitis grows due to the release of ammonia and ammonium ions. The C. immitis urease was purified to homogeneity (1048-fold) from the mycelial cytosol by chromatographic fractionation. The sequence of 12 N-terminal amino-acid residues of the purified, native polypeptide was identical to that predicted by the translated urease gene sequence which has been reported. The isolated enzyme exhibited a specific activity in the presence of urea of 1750 [micro]mol min-1 mg-1 protein, has a native molecular mass of 450 kDa, revealed a Km for urea of 4.1 mM, had a pH optimum of 8.0 and is heat stable. Hydroxyurea, acetohydroxamic acid (AHA) and boric acid each inhibited activity of the **purified enzyme**. Urease activity was enhanced by the presence of 5-10 mM concentrations of Mg2+ or Mn2+, but inhibited by Li+, Ni2+, Cu2+ or Zn2+. The reversible urease inhibitor, AHA, blocked enzyme activity in the crude mycelial cytosolic fraction when added at a concentration of 10 mM. On the other hand, 10 mM AHA added to 4-day-old mycelial cultures only partially decreased the amount of ammonium detected in the culture medium. It is evident, therefore, that C. immitis urease activity does not account for the total amount of ammonia secreted during in vitro growth of the pathogen. Other metabolic sources of ammonia, which may also contribute to the virulence of C. immitis, are under investigation.

L4 ANSWER 13 OF 30 CABA COPYRIGHT 2004 CABI on STN
AN 97:58530 CABA
DN 19970304500
TI Isolation and characterization of two phloroglucinol oxidases from cabbage (Brassica oleracea L.)
AU Fujita, S.; Nazamid, S.; Maegawa, M.; Samura, N.; Hayashi, N.; Tono, T.;

Bin Saari, N.
CS Laboratory of Food Science, Faculty of Agriculture, Saga University, Saga 840, Japan.
SO Journal of Agricultural and Food Chemistry, (1997) Vol. 45, No. 1, pp. 59-63. 30 ref.
ISSN: 0021-8561
DT Journal
LA English
ED Entered STN: 19970612
Last Updated on STN: 19970612
AB Two isozymes (F-IB and F-II) of phloroglucinol oxidase (PhO) were purified from cabbage. The **purified enzymes** were found to be in a homogeneous state by PAGE and SDS-PAGE. The MW of F-IB and F-II were estimated to be 43 000 and 32 000, respectively, by SDS-PAGE. Both **purified enzymes** only oxidized 1,3,5-trihydroxybenzenes, such as phloroglucinol and phloroglucinolcarboxylic acid. Both enzymes also had strong peroxidase (POD) activity. The pH optima of PhO and POD of F-IB were 8.0 and 6.7, respectively, and those of F-II were 7.4 and 6.7, respectively. Activities of both F-IB and F-II were stable in the pH range 6-11 at 5[deg]C for 20 h, and were markedly inhibited by sodium diethyldithiocarbamate and potassium cyanide. MnCl2 markedly activated the PhO activity of F-IB and F-II, but strongly inhibited their POD activity.

L4 ANSWER 14 OF 30 CABA COPYRIGHT 2004 CABI on STN
AN 94:62751 CABA
DN 19940704132
TI Study on the purification and biochemical properties of the soyabean (Glysine max) urease enzyme
Soya fasulyesi (Glysine max) ureaznn saflastrlmas ve baz biyokimyasal ozelliklerinin incelenmesi
AU Guler, G.; Aksoz, E.
SO Doga, Turk Biyoloji Dergisi, (1992) Vol. 16, No. 3, pp. 193-202. 13 ref.
DT Journal
LA Turkish
SL English
ED Entered STN: 19941101
Last Updated on STN: 19941101
AB The soyabean [Glycine max] urease enzyme was purified 28.24 fold compared with the crude enzyme, using ammonium sulphate precipitation, dialysis and DEAE cellulose ion exchange chromatography methods. K+, Hg2+, Cu2+, Fe2+, Zn2+, Ca2+, Mg2+ and Li2+ affected enzyme activity. While 2% gum arabic protected against inhibition of the enzyme, 10-2 M cysteine caused a high level of activation. The activity of the partially **purified enzyme** decreased slightly after storage at 4[deg]C for a week.

L4 ANSWER 15 OF 30 JICST-EPlus COPYRIGHT 2004 JST on STN
AN 910900513 JICST-EPlus
TI Purification and Properties of an alpha-L-Arabinofuranosidase from Cotyledons of Soybean Seedlings.
AU HATANAKA H
IMAOKA H
TAJIMA S; KASAI T
CS Ishikawa Agricultural Coll., Ishikawa, JPN
Environment Dep., Takamatsu City Office, Takamatsu, JPN
Kagawa Univ., Kagawa, JPN
SO Agric Biol Chem, (1991) vol. 55, no. 10, pp. 2599-2605. Journal Code: G0021A (Fig. 4, Tbl. 3, Ref. 21)
CODEN: ABCHA6; ISSN: 0002-1369
CY Japan
DT Journal; Article
LA English
STA New
AB An .ALPHA.-L-arabinosidase(.ALPHA.-L-arabinofuranoside arabinofuranohydrolase, EC 3.2.1.55)that can degrade both purified water

soluble polysaccharide from soybean seeds and p-nitrophenyl-.ALPHA.-L-arabinofuranoside was purified 453-fold to near homogeneity from cotyledons of 4-day-old soybean seedlings. The optimum pH and Km against p-nitrophenyl-.ALPHA.-L-arabinofuranoside of the **purified enzyme** were 4.8 and 0.53mM, respectively. The enzyme was activated by Ca2+ and Zn2+ ions, but Cu2+, Hg2+, EDTA, and L-arabonic-.GAMMA.-lactone inhibited the enzyme activity. The molecular weight of the enzyme subunit was estimated to be 87,000 by SDS-PAGE. (author abst.)

L4 ANSWER 16 OF 30 JICST-EPlus COPYRIGHT 2004 JST on STN
AN 910045235 JICST-EPlus
TI Purification and properties of mannanase from Oerskovia xanthineolytica.
AU SAEKI K; MIYASHITA Y; WATANABE Y; TAMAI Y
CS Ehime Univ., Ehime, JPN
SO J Ferment Bioeng, (1990) vol. 70, no. 4, pp. 215-221. Journal Code: G0535B
(Fig. 9, Tbl. 3, Ref. 33)
CODEN: JFBIEX; ISSN: 0922-338X
CY Japan
DT Journal; Article
LA English
STA New
AB A microorganism, identified as Oerskovia xanthineolytica, which is capable of growing on Saccharomyces cerevisiae mannan as a carbon source has been isolated. When grown on yeast mannan, the microorganism secretes an .ALPHA.-mannanase into the culture medium. The .ALPHA.-mannanase purified by chromatography with phenyl-Sepharose CL-4B, DEAE-Sephacel, and by gel-filtration on Sephacryl S-300 gave a single band on nondenaturing polyacrylamide gel electrophoresis and a single precipitation line with antibodies raised against the **purified enzyme**. The purified .ALPHA.-mannanase could be dissociated into several polypeptides depending on the denaturation conditions of protein. The molecular weight of the enzyme was estimated as over 1,500,000 and the sedimentation coefficient (s20,w) was 16.4s. The enzyme showed optimal activity at pH between 7.0 to 8.0 and optimum temperature at 50.DEG.C.. The enzyme seemed to be Ca2+-dependent. The enzyme was activated by cysteine and sulfhydryl reagents. The enzyme was strongly inactivated by Zn2+, Fe2+, and Cu2+, and completely inhibited by Ni2+, Hg2+, iodoacetic acid, EDTA, and cetyltrimetyl ammonium bromide. The **purified enzyme** hydrolyzed p-nitrophenyl .ALPHA.-mannopyranoside, .ALPHA.-1,2-mannopyranosyl-D-mannopyranose, and .ALPHA.-1,3-mannopyranosyl-D-mannopyranose. The enzyme could also completely disintegrate the antigenic activities of yeast mannan prepared from S. cerevisiae. (author abst.)

L4 ANSWER 17 OF 30 JICST-EPlus COPYRIGHT 2004 JST on STN
AN 900304687 JICST-EPlus
TI Preparation and some properties of a novel maltopentaose-forming enzyme of a Pseudomonas species.
AU KOBAYASHI S
OKEMOTO H; HARA K; HASHIMOTO H
CS National Food Research Inst., Ministry of Agriculture, Forestry and Fisheries, JPN
Ensuiko Sugar Refining Co., Ltd., Yokohama, JPN
SO Agric Biol Chem, (1990) vol. 54, no. 1, pp. 147-156. Journal Code: G0021A
(Fig. 12, Tbl. 3, Ref. 20)
CODEN: ABCHA6; ISSN: 0002-1369
CY Japan
DT Journal; Article
LA English
STA New
AB A novel enzyme, of a Pseudomonas species, was purified by ammonium sulfate fractionation and column chromatographies on DEAE-Toyopearl 650M and Toyopearl HW-55s. The activity recovery was 87% at the ammonium sulfate fractionation step and 44% at the final step of the purification, respectively. The **purified enzyme** was homogeneous

electrophoretically and its molecular weight was 72,000. The optimum pH and temperature were 6.5 and 55.DEG.C., respectively. The enzyme was stable up to 45.DEG.C. in the pH range of 6.5 to 9.0 for 1hr, and thermostable in the presence of 2mM CaCl2 up to 50.DEG.C.. The isoelectric point of the enzyme was 6.5. The enzyme activity was inhibited by metal ions such as Zn2+, Cu2+, Ag+ and Hg2+, and enhanced by Rb+, Mg2+, Co2+ and Ca2+. The enzyme specifically produced maltopentaose from starch in the initial stage of the reaction,finally producing maltose and maltotriose. The enzyme did not act on glucose, maltose or maltotriose, and maltohexaose was considerably resistant to the enzyme's action. In the initial stage of hydrolysis, G2 and G5 were formed from G7, and thus G3+G5 from G8, G4+G5 from G9, G5+G5 from G10, and G6+G5 from G11. Through the next action, G4 and G5 were degraded to G2+G2 and G2+G3, respectively. The enzyme action proceeds over the branching points of amylopectin to produce large amounts of G2 and G3, and a residual amount of oligosacchfriden having lenn than 10 glucose units in the final stage of the reaction. (author adst.)

L4 ANSWER 18 OF 30 JICST-EPlus COPYRIGHT 2004 JST on STN
AN 880286812 JICST-EPlus
TI Purification and properties of two .BETA.-glucosidases from Penicillium herquei Banier and Sartory.
AU FUNAGUMA T; HARA A
CS Meijo Univ., Nagoya, JPN
SO Agric Biol Chem, (1988) vol. 52, no. 3, pp. 749-755. Journal Code: G0021A (Fig. 5, Tbl. 4, Ref. 26)
CODEN: ABCHA6; ISSN: 0002-1369
CY Japan
DT Journal; Article
LA English
STA New
AB Two .BETA.-glucosidases, G1 and G2, were purified from the culture supernatant of Penicillium herquei Banier and Sartory. Both the **purified enzymes** were homogeneous on polyacrylamide disc gel electrophoresis. The molecular weights of G1 and G2 were estimated to be 125,000 and 122,000, respectively, by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. G1 and G2 contained 12.7% and 16.1% carbohydrate as glucose, and had isoelectric points of 5.02 and 5.24, respectively. Both enzymes had optimum pHs of 4.0-4.5 and optimum temperatures at 60.DEG.C, but pH- and thermo-stabilities of G1 were higher than those of G2. Both enzymes were active not only on p-nitrophenyl .BETA.-D-glucopyranoside, salicin, and the .BETA.-glucobioses tested but also on laminarin. CM-Cellulose was a very poor substrate for both enzymes. The activities of G1 toward the substrates except for laminarin and CM-cellulose were apparently higher than those of G2. Both enzymes acted on cellobiose to produce a transfer product.(author abst.)

L4 ANSWER 19 OF 30 MEDLINE on STN
AN 2003190854 MEDLINE
DN PubMed ID: 12628378
TI Purification and characterization of cathepsin L in arrowtooth flounder (Atheresthes stomias) muscle.
AU Visessanguan Wonnop; Benjakul Soottawat; An Haejung
CS BIOTEC--Central Research Unit, National Center for Genetic Engineering and Biotechnology, 113 Paholayothin Road, Klong 1, Klong Laung, Patumthani 12120, Thailand.. wonnop@biotec.or.th
SO Comparative biochemistry and physiology. Part B, Biochemistry & molecular biology, (2003 Mar) 134 (3) 477-87.
Journal code: 9516061. ISSN: 1096-4959.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200312

ED Entered STN: 20030425
Last Updated on STN: 20031224
Entered Medline: 20031223

AB A predominant, heat-activated proteinase in muscle extract of arrowtooth flounder (Atheresthes stomias) was purified to 55-fold by heat treatment, followed by a series of chromatographic separations. The apparent molecular mass of the **purified enzyme** was 27 kDa by size exclusion chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteinase had high affinity and activity toward Z-Phe-Arg-NMec with K(m) and k(cat) values of 8.2 microM and 12.2/s, respectively. Activity was inhibited by sulfhydryl reagents and activated by reducing agents. The purified proteinase displayed optimal activity at pH 5.0-5.5 and 60 degrees C, respectively. Consistent with the properties of proteases from other species, the heat-activated proteinase in arrowtooth flounder can be identified as cathepsin L.

L4 ANSWER 20 OF 30 MEDLINE on STN
AN 2001178536 MEDLINE
DN 21106294 PubMed ID: 11160856
TI Molecular mechanisms involved in the synergistic activation of soluble guanylyl cyclase by YC-1 and nitric oxide in endothelial cells.
AU Schmidt K; Schrammel A; Koesling D; Mayer B
CS Institut fur Pharmakologie und Toxikologie, Karl-Franzens-Universitat Graz, Graz, Austria.. kurt.schmidt@kfunigraz.ac.at
SO MOLECULAR PHARMACOLOGY, (2001 Feb) 59 (2) 220-4.
Journal code: 0035623. ISSN: 0026-895X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200103
ED Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010329

AB YC-1 is a direct activator of soluble guanylyl cyclase (sGC) and sensitizes the enzyme for activation by nitric oxide (NO) and CO. Because the potentiating effect of YC-1 on NO-induced cGMP formation in platelets and smooth muscle cells has been shown to be substantially higher than observed with the **purified enzyme**, the synergism between heme ligands and YC-1 is apparently more pronounced in intact cells than in cell-free systems. Here, we investigated the mechanisms underlying the synergistic activation of sGC by YC-1 and NO in endothelial cells. Stimulation of the cells with YC-1 enhanced cGMP accumulation up to approximately 100-fold. The maximal effect of YC-1 was more pronounced than that of the NO donor DEA/NO (approximately 20-fold increase in cGMP accumulation) and markedly diminished in the presence of L-N(G)-nitroarginine, EGTA, or oxyhemoglobin. Because YC-1 did not activate endothelial NO synthase, the pronounced effect of YC-1 on cGMP accumulation was apparently caused by a synergistic activation of sGC by YC-1 and basal NO. The effect of YC-1 was further enhanced by addition of DEA/NO, resulting in a approximately 160-fold stimulation of cGMP accumulation. Thus, YC-1 increased the NO-induced accumulation of cGMP in intact cells by approximately 8-fold. Addition of endothelial cell homogenate increased the stimulatory effect of YC-1 on NO-activated purified sGC from 1.2- to 3.7-fold. This effect was not observed with heat-denatured homogenates, suggesting that a heat-labile factor present in endothelial cells potentiates the effect of YC-1 on NO-activated sGC.

L4 ANSWER 21 OF 30 PROMT COPYRIGHT 2004 Gale Group on STN

AN 2002:467781 PROMT
TI Chemical tradenames. (F-P).(list of chemical companies throughout the world with contact data)(Industry Overview)(Cover Story)
SO Chemical Week, (27 Sep 2002) Vol. 164, No. 38, pp. 486(12).

ISSN: ISSN: 0009-272X.
PB Chemical Week Associates
DT Newsletter
LA English
WC 18020
FULL TEXT IS AVAILABLE IN THE ALL FORMAT
AB F-1000, 2000, 2100, 2200, 2300, 3600, 4400: Aluminum hydroxide dried gel -- Reheis Inc

Subscription: $99.00 per year. Published weekly. P.O. Box 7721, Riverton, NJ 08077-9021.

L4 ANSWER 22 OF 30 USPATFULL on STN
AN 2001:75169 USPATFULL
TI Biocatalysts with amine acylase activity
IN Ghisalba, Oreste, Reinach, Switzerland
Kittelmann, Matthias, Freiburg, Germany, Federal Republic of
Laumen, Kurt, March, Germany, Federal Republic of
Walser-Volken, Paula, Ziefen, Switzerland
PA Novartis AG, Basel, Switzerland (non-U.S. corporation)
PI US 6235516 B1 20010522
WO 9741214 19971106
AI US 1998-171646 19981112 (9)
WO 1997-EP1866 19970414
19981112 PCT 371 date
19981112 PCT 102(e) date
PRAI EP 1996-810266 19960425
DT Utility
FS Granted
EXNAM Primary Examiner: Achutamurthy, Ponnathapu; Assistant Examiner: Rao, Manjunath
LREP Ferraro, Gregory D.
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1804
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention belongs to the field of biotechnology. It concerns a biocatalyst, i.e. a dead or living microorganism or a polypeptide, preferably in isolated form, which exhibits acylase enzymatic activity without lipase- or esterase-activity. The biocatalyst is capable of stereoselectively hydrolysing a racemic acylamide which has an aliphatic acyl residue and which is not a derivative of a natural amino acid.

L4 ANSWER 23 OF 30 USPATFULL on STN
AN 1999:21929 USPATFULL
TI Method for determining activity of enzymes in metabolically active whole cells
IN Lucas, Frank J., Boca Raton, FL, United States
Jaffe, Gerald E., Pembroke Pines, FL, United States
Bott, Steven, Pembroke Pines, FL, United States
Carter, James H., Plantation, FL, United States
PA Coulter Corporation, Miami, FL, United States (U.S. corporation)
PI US 5871946 19990216
AI US 1995-444056 19950518 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Gitomer, Ralph
LREP Alter, Mitchell E.
CLMN Number of Claims: 32
ECL Exemplary Claim: 1
DRWN 50 Drawing Figure(s); 45 Drawing Page(s)
LN.CNT 4547

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An assay compound or a salt thereof for assaying the activity of an enzyme inside a metabolically active whole cell is disclosed. The assay compound includes a leaving group and an indicator group. The leaving group is selected from the group comprising amino acids, peptides, saccharides, sulfates, phosphates, esters, phosphate esters, nucleotides, polynucleotides, nucleic acids, pyrimidines, purines, nucleosides, lipids and mixtures thereof. The indicator group is selected from compounds which have a first state when joined to the leaving group, and a second state when the leaving group is cleaved from the indicator group by the enzyme. Preferably, the indicator compounds are rhodamine 110, rhodol, and fluorescein and analogs of these compounds. A method of synthesizing the compound as well as methods of using these compounds to measure enzyme activity are also disclosed.

L4 ANSWER 24 OF 30 USPATFULL on STN
AN 1998:157128 USPATFULL
TI Assay reagent
IN Jaffe, Gerald E., Pembroke Pines, FL, United States
Lucas, Frank J., Boca Raton, FL, United States
Carter, James H., Plantation, FL, United States
PA Coulter Corporation, Miami, FL, United States (U.S. corporation)
PI US 5849513 19981215
AI US 1997-904400 19970731 (8)
RLI Division of Ser. No. US 1995-443776, filed on 18 May 1995
DT Utility
FS Granted
EXNAM Primary Examiner: Gitomer, Ralph
LREP Alter, Mitchell E.
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 50 Drawing Figure(s); 45 Drawing Page(s)
LN.CNT 4370
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An assay compound or a salt thereof for assaying the activity of an enzyme inside a metabolically active whole cell is disclosed. The assay compound includes a leaving group and an indicator group. The leaving group is selected from the group comprising amino acids, peptides, saccharides, sulfates, phosphates, esters, phosphate esters, nucleotides, polynucleotides, nucleic acids, pyrimidines, purines, nucleosides, lipids and mixtures thereof. The indicator group is selected from compounds which have a first state when joined to the leaving group, and a second state when the leaving group is cleaved from the indicator group by the enzyme. Preferably, the indicator compounds are rhodamine 110, rhodol, and fluorescein and analogs of these compounds. A method of synthesizing the compound as well as methods of using these compounds to measure enzyme activity are also disclosed.

L4 ANSWER 25 OF 30 USPATFULL on STN
AN 1998:78969 USPATFULL
TI Assay reagent
IN Jaffe, Gerald E., Pembroke Pines, FL, United States
Lucas, Frank J., Boca Raton, FL, United States
Carter, James H., Plantation, FL, United States
PA Coulter Corporation, Miami, FL, United States (U.S. corporation)
PI US 5776720 19980707
AI US 1995-443776 19950518 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Gitomer, Ralph
LREP Alter, Mitchell E.
CLMN Number of Claims: 47
ECL Exemplary Claim: 1
DRWN 50 Drawing Figure(s); 45 Drawing Page(s)

LN.CNT 4423
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An assay compound or a salt thereof for assaying the activity of an enzyme inside a metabolically active whole cell is disclosed. The assay compound includes a leaving group and an indicator group. The leaving group is selected from the group comprising amino acids, peptides, saccharides, sulfates, phosphates, esters, phosphate esters, nucleotides, polynucleotides, nucleic acids, pyrimidines, purines, nucleosides, lipids and mixtures thereof. The indicator group is selected from compounds which have a first state when joined to the leaving group, and a second state when the leaving group is cleaved from the indicator group by the enzyme. Preferably, the indicator compounds are rhodamine 110, rhodol, and fluorescein and analogs of these compounds. A method of synthesizing the compound as well as methods of using these compounds to measure enzyme activity are also disclosed.

L4 ANSWER 26 OF 30 USPATFULL on STN
AN 1998:33749 USPATFULL
TI Method of making an assay compound
IN Jaffe, Gerald E., Pembroke Pines, FL, United States
Lucas, Frank J., Boca Raton, FL, United States
Carter, James H., Plantation, FL, United States
PA Coulter Corporation, Miami, FL, United States (U.S. corporation)
PI US 5733719 19980331
AI US 1995-445217 19950518 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Gitomer, Ralph
LREP Alter, Mitchell E.
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 50 Drawing Figure(s); 45 Drawing Page(s)
LN.CNT 4393
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An assay compound or a salt thereof for assaying the activity of an enzyme inside a metabolically active whole cell is disclosed. The assay compound includes a leaving group and an indicator group. The leaving group is selected from the group comprising amino acids, peptides, saccharides, sulfates, phosphates, esters, phosphate esters, nucleotides, polynucleotides, nucleic acids, pyrimidines, purines, nucleosides, lipids and mixtures thereof. The indicator group is selected from compounds which have a first state when joined to the leaving group, and a second state when the leaving group is cleaved from the indicator group by the enzyme. Preferably, the indicator compounds are rhodamine 110, rhodol, and fluorescein and analogs of these compounds. A method of synthesizing the compound as well as methods of using these compounds to measure enzyme activity are also disclosed.

L4 ANSWER 27 OF 30 USPATFULL on STN
AN 97:117905 USPATFULL
TI Method for determining activity of enzymes in metabolically active whole cells
IN Lucas, Frank J., Baca Raton, FL, United States
Jaffe, Gerald E., Pembroke Pines, FL, United States
Bott, Steven E., Pembroke Pines, FL, United States
Carter, James H., Plantation, FL, United States
PA Coulter Corporation, Miami, FL, United States (U.S. corporation)
PI US 5698411 19971216
AI US 1995-444051 19950518 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Gitomer, Ralph
LREP Alter, Mitchell E.
CLMN Number of Claims: 77

ECL Exemplary Claim: 1
DRWN 50 Drawing Figure(s); 45 Drawing Page(s)
LN.CNT 4867
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method for assaying the activity of an enzyme inside a metabolically active whole cell is disclosed. The assay compound includes a leaving group and an indicator group. The leaving group is selected from the group comprising amino acids, peptides, saccharides, sulfates, phosphates, esters, phosphate esters, nucleotides, polynucleotides, nucleic acids, pyrimidines, purines, nucleosides, lipids and mixtures thereof. The indicator group is selected from compounds which have a first state when joined to the leaving group, and a second state when the leaving group is cleaved from the indicator group by the enzyme. Preferably, the indicator compounds are rhodamine 110, rhodol, and fluorescein and analogs of these compounds. A method of synthesizing the compound is also disclosed.

L4 ANSWER 28 OF 30 USPATFULL on STN
AN 87:46996 USPATFULL
TI Clam derived proteinases
IN Chen, Hung-Chang, Elmsford, NY, United States
Zall, Robert R., Ithaca, NY, United States
PA Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation)
PI US 4677069 19870630
AI US 1984-683108 19841218 (6)
DT Utility
FS Granted
EXNAM Primary Examiner: Naff, David M.
LREP Jones, Tullar & Cooper
CLMN Number of Claims: 5
ECL Exemplary Claim: 1,3
DRWN 9 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 2689
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to three enzymes, their isolation from the viscera of bivalves, e.g. the surf clam or cherrystone clam, their characterization and uses. The first two are carboxyl proteinases having molecular weights of about 77,200 and about 36,700 and display activity similar to mammalian D-cathepins. The third is a thiol proteinase having a molecular weight of about 17,400 and displays activity similar to mammalian B-cathepins. In addition to attaching various substrates, the enzymes coagulate cheese milk and tenderize meat.

L4 ANSWER 29 OF 30 USPATFULL on STN
AN 77:47844 USPATFULL
TI Method for detecting enzymes capable of digesting fibrinogen or fibrin
IN Moroz, Leonard Arthur, Westmount, Canada
PA Canadian Patents and Development Limited, Ottawa, Canada (non-U.S. corporation)
PI US 4046635 19770906
AI US 1975-608237 19750827 (5)
DT Utility
FS Granted
EXNAM Primary Examiner: Tanenholtz, Alvin E.; Assistant Examiner: Fan, C. A.
LREP Cushman, Darby & Cushman
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 466
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method for detecting and measuring the activity of enzymes capable of digesting fibrinogen or fibrin and for detecting and measuring the activity of **enzyme activators** and **enzyme**

inhibitors of said enzymes, by incubating an unknown enzyme sample in a humid labelled fibrinogen or labelled fibrin coated test apparatus, isolating the labelled degradation products of fibrinogen or fibrin released by the unknown enzyme sample and measuring the labelled degradation products of fibrinogen released by the unknown sample.

L4 ANSWER 30 OF 30 WPINDEX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2003-120408 [11] WPINDEX
DNN N2003-095987 DNC C2003-031000
TI Reporter system for detecting analyte in liquid, comprises solid supports containing immobilized capture molecules, and immobilized inactive enzyme molecules, **enzyme activator**-containing affinity liposomes, and substrates.
DC A89 B04 D16 S03
IN BREDEHORST, R; HEUBERGER, A; HINTSCHE, R
PA (FRAU) FRAUNHOFER GES FOERDERUNG ANGEWANDTEN
CYC 21
PI WO 2002082078 A2 20021017 (200311)* EN 150p
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: JP US
ADT WO 2002082078 A2 WO 2002-EP3890 20020408
PRAI US 2001-282088P 20010409
AB WO 200282078 A UPAB: 20030214
NOVELTY - A reporter system (I) for detecting an analyte (A) present in a liquid, comprises a solid support (1) containing immobilized capture molecules (2), and a solid support containing immobilized inactive enzyme molecules, **enzyme activator**-containing affinity liposomes, and substrates for detection procedures.

DETAILED DESCRIPTION - A reporter system (I) for detecting (A) present in a liquid, comprises the following components:

(i) capture molecules (C) immobilized on a solid support (SS) and capable of specifically binding (A);

(ii) **enzyme-activator** containing affinity components, chosen from:

(i) affinity liposomes (II) containing encapsulated **enzyme activator** and comprising a surface-attached affinity component capable of specifically binding to (A) and/or the (C) in a condition, where (A) and (C) are bound to each other, but not to free (C);

(ii) polymeric carrier molecules containing a covalently linked affinity component capable of specifically binding to (A) and/or (C) in a condition where (A) and (C) are bound to each other, but not to free (C), and two covalently linked affinity components capable of specifically binding to affinity liposomes, plus affinity liposomes (III) which contain encapsulated **enzyme activator** and comprising a surface-attached affinity component capable of specifically binding to the polymeric carrier molecules;

(iii) complexes of (II); or

(iv) polymeric carrier molecules containing a covalently linked affinity component capable of specifically binding to (A) and/or (C) in a condition where (A) and (C) are bound to each other, but not to free (C), and more than one covalently linked affinity components capable of specifically binding to affinity liposomes, plus the complexes of (III);

(iii) inactive enzyme molecules immobilized on a SS and capable of being activated by the **enzyme activator** encapsulated in the affinity liposomes; and

(iv) a substrate not detectable by the reporter system which in the presence of activated enzyme molecules is convertible into reporter molecules capable of being detected by the reporter system.

USE - (I) is useful for detecting an analyte (antigens, antibodies, nucleic acids or amplicons) in a liquid sample, which involves:

(a) providing (I);

(b) contacting the sample with the immobilized (C);

(c) adding the **enzyme activator**-containing affinity liposomes;

(d) removing unbound affinity liposomes;

(e) releasing encapsulated **enzyme activator** from the interior of affinity liposomes bound to (A) and/or (C);

(f) bringing the immobilized, inactive enzyme molecules into contact with **enzyme activator** released from the interior of the affinity liposomes and restoring the activity of the enzyme molecules;

(g) adding the substrate being convertible into reporter molecules to the enzyme molecules; and

(h) measuring the reporter molecules formed by activated enzyme molecules.

Preferably, the **enzyme activator** is released from the interior of the liposome by increasing the ambient temperature or by adding a liposome-lysing agent. The method further involves adding polymeric carrier molecules capable of specifically binding to (A) and/or (C) in conditions where (A) and (C) are bound to each other but not to free (C), the polymeric carrier molecules being the components bound or later to be bound to the affinity liposomes by providing more than one binding site for the affinity liposomes per each of the polymeric carrier molecules and removing unbound polymeric carrier molecules, before adding **enzyme activator** containing affinity liposomes, and where the affinity liposomes added are capable of specifically binding to (A)-bound polymeric carrier molecules. Optionally, detecting an analyte (preferably a low molecular weight (A)) in liquid sample comprises:

(a) providing (I) comprising (C) immobilized on a solid support and capable of specifically binding (A), affinity liposomes containing encapsulated **enzyme activator** and having (A) to be analyzed attached to their surface, enzyme molecules in an inactive condition immobilized on a solid support, and a substrate being convertible to reporter molecules by the enzyme molecules in activated condition;

(b) mixing the sample with a limited quantity of the affinity liposomes;

(c) contacting the mixture with the immobilized (C);

(d) removing unbound affinity liposomes;

(e) releasing encapsulated **enzyme activator** from the interior of affinity liposomes bound to (C);

(f) bringing the immobilized, inactive enzyme molecules into contact with **enzyme activator** released from the interior of the affinity liposomes and restoring the activity of the enzyme molecules;

(g) adding the substrate being convertible into reporter molecules to the enzyme molecules; and

(h) measuring the reporter molecules formed by activated enzyme molecules.

Alternatively, detecting (A) in a liquid sample, involves:

(a) providing (C) immobilized on a solid support and capable of specifically binding (A), (A) molecules labeled with an affinity component capable of binding affinity liposomes containing surface-attached complementary affinity components, affinity liposomes containing encapsulated enzyme activator and having the complementary affinity components attached to their surface, enzyme molecules in an inactive condition immobilized on a solid support, a substrate being convertible to reporter molecules via the enzyme molecules in activated condition;

(b) mixing the sample with a limited quantity of (A) molecules;

(c) contacting the mixture with the immobilized (C);

(d) adding affinity liposomes;

(e) removing unbound affinity liposomes;

(f) releasing encapsulated enzyme activator from the interior of affinity liposomes bound to labeled (A); and

(g) carrying out steps (f)-(h) of the previous method (all claimed).

ADVANTAGE - The enzymatic amplification system allows signal enhancement by several orders of magnitude. Since affinity liposomes allow encapsulation of large amounts of enzyme activators, multiple enzyme molecules are activated upon release of the enzyme activators from each (A)-bound affinity liposome. Only partial purified enzyme preparations are

used, and the size of the solid-phase area can be easily adjusted to accommodate additional contaminating, enzymatically inactive proteins. As a result (I) reduces costs and provides a new technique for highly efficient signal amplification.

DESCRIPTION OF DRAWING(S) - The figure shows detection of target nucleic acids using affinity liposomes containing encapsulated Zn2+ ions on surface attached intercalating agents.

Solid support 1

Surface bound capture oligonucleotide 2

Dwg.1A/2